Bacteria but not fungi communities differ among perennial grassland ecotypic rhizosphere under abiotic environmental stress

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Abstract

Environmental change, especially frequent droughts, is predicted to detrimentally impact the North American perennial grasslands. Consistent dry spells will affect plant communities as well as their associated rhizobiomes, possibly altering the plant host performance under environmental stress. Therefore, there is a need to understand the impact of drought on the rhizobiome, and how the rhizobiome may modulate plant host performance and ameliorate response to drought stress. In this study, we investigated the differences in bacterial and fungal community composition between three ecotypes (dry, mesic, and wet) of a dominant prairie grass, *Andropogon gerardii*. The ecotypes were established in 2010 in a common garden setup and grown under long-term dry conditions at the arid margin of the species' range in Colby KS. The main question to be answered was to what extent do the wet and dry ecotypes maintain or recruit distinct rhizobiomes after ten years of growing in an arid climate and do these rhizobiomes ameliorate drought stress? In order to answer this question, we screened the ecotype rhizobiome profiles under the arid growing conditions of western KS as a surrogate for future climate environmental stress using 16S rRNA and ITS-1 amplicon sequencing. Under these conditions, bacterial community structure differed among the *A. gerardii* ecotypes, whereas the fungal communities did not differ compositionally. The ecotypes were instrumental in driving the differences among rhizobiome bacterial structures, further maintaining ecotype distinct bacterial rhizobiomes even after inhabiting a dry environment for ten years. This study will aid us to optimize plant productivity through the use of different ecotypes under future abiotic environmental stress, especially drought.

Key words
Rhizobiome, bacteria, fungi, drought, ecotypes, grass

Introduction

The rhizosphere, which plays a pivotal role in plant function by facilitating elemental and water cycling, and trapping absorption of nutrients (Adl 2016), is densely populated with diverse microorganisms. A wide range of complex interactions ranging from symbiotic to competitive among the rhizosphere-inhabiting microorganisms, collectively termed the rhizobiome, govern the carbon, nitrogen, and phosphorus processes uptake and transformations (Ahkami et al. 2017; Barea et al. 2005). These interactions exist not only among the microorganisms but also between the plant hosts and their associated rhizobiome. For example, plant hosts may selectively attract and/or repel specific soil microbial communities through their root exudates (Bais et al. 2001; Estabrook and Yoder 1998). Furthermore, these microorganisms may establish symbiotic relationships with the host plants, safeguarding the plant systems against pathogenic entities (Nardi et al. 2000; Jones et al. 2019). These interactions between the plant host and its rhizobiome are likely to be highly specific and ultimately important for community stability, ecosystem functioning related to biogeochemical cycling, and maintaining soil biodiversity (Wagg et al. 2021).

Global climatic change can have an adverse effect on such microbe-microbe and plant-microbe interactions, which in turn, can impact the ecology of the rhizosphere and ecosystem function (Ahkami et al. 2017). Some studies have focused on the impacts of climatic change on rhizospheres (Classen et al. 2015; Bei et al. 2019; Compant, Van Der Heijden, and Sessitsch 2010). However, more concerted efforts are needed to answer the knowledge gaps between how rhizobiomes may be influenced by the interactive effects of the plant host and the ever-changing climatic patterns. Ultimately, This will help us to maximize plant growth and survival in stressful environments (Long and Zhu 2009; Long, Marshall-Colon, and Zhu 2015; Ort et al. 2015), especially impacts from drought.

Microorganisms in the rhizosphere are highly sensitive to environmental conditions and can be good indicators of soil quality (Mendes, Garbeva, and Raaijmakers 2013). Identifying the rhizosphere bacterial and fungal communities, their functional roles, and their interactions with the plant-hosts are crucial to developing future methods to improve drought tolerance and provide tools for environmental conservation. Also, comprehending the extent of the variability among ecotypes of the dominant species and how this variability interacts under the predicted arid conditions is important. This is especially critical when the dominant species greatly impact ecosystem processes such as carbon assimilation, nutrient cycling etc. (Grime 1998). The drive to understand plant host-microbe interaction is even more crucial in the case of tallgrass prairies, in which dominant grasses are responsible for the majority of the carbon fixing, nutrient cycling and biomass (Risser, Birney, and Blocker 1981; Johnson and Matchett 2001).

Our focal species is Big Bluestem, *Andropogon gerardii*, the dominant native grass species of the tallgrass prairies of central North America (Gustafson, Gibson, and Nickrent 2004). *Andropogon gerardii* is widely distributed across the Midwest and Northeastern US (USDA database). Our study focuses on the Central grasslands where this grass dominates, stretching from western Kansas (rainfall 500 mm/year) to southern Illinois (1,200 mm/year) (Johnson et al. 2015). This precipitation gradient includes a drought-like environment (Colby 500 mm/year and Hays, KS 580 mm/year), a region of intermediate precipitation (Manhattan, KS 870 mm/year), and a region of heavy rainfall (Carbondale, IL 1160 mm/year) (Olsen et al. 2013). Climate change models have also depicted a strong phenotypic cline in *A. gerardii* across this longitudinal precipitation gradient (Galliart et al. 2019, 2020; Smith et al. 2017). Galliart and colleagues have further demonstrated that within this precipitation gradient, three genetically distinct *A. gerardii* regional climate ecotypes (dry, mesic, and wet) exist (Gray et al. 2014; Galliart et al. 2019), in

terms of leaf area, height, and blade width (Galliart et al. 2020), allocation to roots (Mendola et al. 2016), and chlorophyll abundance (Caudle et al. 2014). However, how the extreme environmental conditions – the drought-like environment of Colby, KS – may affect the plant ecotypes and their rhizobiome remains unknown. Colby represents the westernmost edge of Kansas where *A. gerardii* occurs only in isolated patches. As such, understanding the shifts and interaction between the *A. gerardii* plant host and its associated microbiome at Colby will provide insights into exploring the impact of future climate change on grasslands and the microbiome.

The goal of this study was to analyze the composition of bacterial and fungal communities in the rhizobiomes of the dry, mesic and wet *A. gerardii* ecotypes originating in Hays, Manhattan KS and Carbondale Illinois (respectively). All ecotypes were planted and grown in Colby for 10 years prior to sampling. The main question we addressed was to what extent do ecotypes of a dominant prairie grass maintain or recruit distinct rhizobiomes after ten years of growing in an arid climate at the edge of their species range and do these rhizobiomes ameliorate drought stress? After ten years, we should be able to see a linkage, if there is one, between host ecotype and rhizobiome.

We were specifically interested in deciphering the extent of ecotypic variation and/or environmental pressure on the dominant tall-grass prairie grass. We postulated that the plant host would exert their ecotypic influences on the rhizobiome even under environmental stress, and thus would observe differences in the ecotypic microbial community. This study aims to provide insights necessary to preserve the prairie ecosystems under climate change pressures.

Materials and Methods

Experimental design and sampling

We utilized a common garden established in 2010 - ten years before our sampling. The seeds of native dry, mesic and wet *A. gerardii* ecotypes were germinated and then grown inside the greenhouse using the potting mix (Metro-Mix 510). After the initial growth, 3-4-month-old plants from all the populations had been planted in western Kansas (Colby rainfall 500 mm/year) as a surrogate for the drier conditions expected in the future (Galliart et al. 2020). Each ecotype was represented by four populations containing twelve plants (Galliart et al. 2020). The Colby garden is located at the KSU Agricultural Research Center in Thomas County 39°23′N, 101°04′W. There were a total of 12 plants (4 populations X 3 ecotypes) in a randomly complete block design with 10 blocks (Galliart et al. 2020). Plants were placed 50 cm apart along each row, and the soil around the plants were covered with a water-penetrable cloth to prevent the growth of any competing plant. We collected a total of 95 rhizosphere soil cores (15 cm deep) from the dry (n=33), mesic (n=30) and wet (n=32) ecotypes during the growing season in Summer 2019. Each soil core was placed in a plastic bag, transported on ice and stored at -80°C until microbial DNA extraction.

DNA extraction, metabarcoding and analyses

We extracted the genomic DNA from the root samples and soil associated with it (0.150g each) using the Omega E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA), with a slightly modified protocol. Any soil that was attached to the root was considered part of the rhizosphere. Briefly, we modified the protocol using a QIAGEN TissueLyser II (Qiagen, Hilden, Germany) for 2 minutes at 20 rev/s, and eluted the purified DNA with a final volume of 100 µl. The extracted microbial DNA were sequenced on the Illumina MiSeq, with the 16S rRNA V4 region amplified using the primers 515F and 806R with barcodes (Caporaso et al. 2012), and the

internal transcribed spacer region ITS2 amplified using the primers ITS4 and fITS7 (Ferrer et al. 2001; Kumeda and Asao 1996; Ihrmark et al. 2012) (Illumina, San Diego, CA, USA), at the Kansas State University Integrated Genomics Facility.

We acquired a total of 1,729,418 bacterial and 3,570,536 fungal sequences before quality check (QC) and trimming (Supplementary Table S1). We used QIIME 2 (v. 2019.7) to process the sequence data and to profile the rhizobiome communities (Bolyen et al. 2019). We used QIIME 2 plugin cutadapt (Martin 2011) to remove the primer sequences; reads with no primer were discarded. Additionally we used DADA2 (Callahan et al. 2016) for quality control with the same parameters across different runs, and truncated the reads to length where the 25th percentile of the reads had a quality score below 15. The pre-trained classifier offered by QIIME 2, using Silva database (v. 132) was used for taxonomic assignment for bacteria. Similarly, the UNITE classifier was trained on the full reference "develop" sequences (version 8.2, release date 2020-2-20) using QIIME 2 2020.2 before taxonomic assignment of the fungal reads. α-diversity was calculated to present the species diversity in each sample (Consortium and The Human Microbiome Project Consortium 2012). We used the Shannon, Faith PD and observed otus indices on a rarefield dataset (8,023 reads for 16S, 10,258 reads for ITS) to measure the number of species and to evaluate the species diversity. Similar to the α-diversity analysis, we used UniFrac and Bray Curtis to evaluate the community dissimilarity patterns in different ecotypes, and used Non-metric multidimensional scaling (NMDS) to distance matrices among different ecotypes.

Differences in the relative abundances of the phyla between the ecotypes was analyzed using ANOVA in R followed by Tukey's post-hoc test ($p < 0.05$). SIMPER post-hoc analyses were performed to identify those community members that most contributed to differences among the ecotypes. The cut-off used for all SIMPER analyses was 70%. We used DeSEQ2 and highlighted

marked differences in the predominance of bacterial communities among the ecotypes ($p < 0.05$) (Love, Huber, and Anders 2014). All raw sequence data is available in the NCBI under the bioproject PRJNA 772708 and biosamples (SAMN 22405120 - SAMN 22405309).

Results and Discussion

We dissected the rhizosphere bacterial and fungal communities associated with the dominant prairie tallgrass, *A. gerardii*. We recovered an average of 17,181 $\pm$ 4,935 amplicon sequence variants (ASVs) per sample for bacteria, and 37,015 $\pm$ 7,394 ASVs for fungal populations after primer trimming (Supplementary Table S1). Of the recovered ASVs, an average of 70.22% bacterial and 61.55% fungal reads were annotated to the species level on SILVA and UNITE respectively. Any ASVs that were marked as unknown or unclassified were removed from further downstream analyses. The bacterial and fungal taxon assignments along with the counts in each sample are provided in Supplementary Table S2.

No differences in bacterial or fungal α-diversity among host ecotypes

The diversity indices (Shannon index, Faith PD index and observed OTUs index) provided no support for differences among ecotypes in terms of bacterial α-diversity. The Shannon index shows the diversity within a sample, while the Faith PD index is a measure of biodiversity, based on phylogeny, and the observed OTUs index represents the richness. Overall, the bacterial diversity (Shannon index: $H = 6.374$, $p = 0.041$; Faith PD index: $H = 3.626$, $p = 0.163$; observed OTUs: $H = 3.959$, $p = 0.138$) did not differ among the ecotypes (Figure 1A). Venn diagrams representing the shared bacterial and archaeal phyla between datasets reveal 1,117,360 shared ASVs among the three ecotypic rhizobiomes (Figure 1A). Predominant phyla belonging to these ASVs were

Actinobacteria, Proteobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, Thaumarchaeota, Chloroflexi, Firmicutes, Patescibacteria, Planctomycetes, Armatimonadetes, Gemmatimonadetes, Latescibacteria, Cyanobacteria, Rokubacteria, Entotheonellaeota, Nitrospirae, BRC1, Chlamydiae, Dependentiae, FBP, Elusimicrobia, Deinococcus-Thermus, Fibrobacteres, and WS2. Similar to the bacterial α-diversity, we also did not find any significant differences in the fungi α-diversity among the ecotypic rhizobiomes. We did not observe any statistical significance in either the Shannon index ($H = 3.759$, $p = 0.153$), faith-pd index ($H = 4.798$, $p = 0.091$) or the observed OTUs index ($H = 3.393$, $p = 0.183$) (Figure 1B). The predominant phyla that overlapped among the dry, wet and mesic ecotypic rhizobiome belonged to Basidiomycota, Ascomycota, Mortierellomycota, Glomeromycota, Kickxellomycota, Chrytridiomycota, Rozellomycota, Aphelidiomycota, and Entomophthoromycota. There were 52 unique fungi ASVs belonging to the mesic ecotypes, while there were 2,129,155 overlapping ASVs among the three ecotypes (Figure 1B).

Bacterial β-Diversity in the rhizobiome differs among host ecotype ecotypic rhizobiome

As shown in Figure 2A, the β-diversity comparisons using NMDS analysis found that bacterial (NMDS; stress 0.13) community compositions are separated among the three ecotypes at the phylum level. We analyzed the samples based on Bray-Curtis similarity, and noticed that the mesic ecotypes showed more similarity among the samples, whereas there existed larger differences in the bacterial community composition among the wet and dry ecotypic samples (Figure 2A). On the other hand, we did not find any distinct clustering pattern of fungi composition among the dry, wet and mesic ecotypic rhizobiomes (NMDS; stress 0.11; Figure 2B).

Significant differences in ecotypic bacterial diversity and structure

We observed that the ecotypic bacterial community compositions were significantly different from each other at the phylum level (PERMANOVA, Pseudo-F 4.1963, , p (perm): 0.001, p (MC): 0.002).

Based on pairwise Kruskal-Wallis test, we detected statistically significant differences in bacterial composition between all the ecotypes (dry v wet: p (MC): 0.034, dry v mesic: p (MC): 0.001, wet v mesic: p (MC): 0.005). The top seven bacterial taxa present in all the three ecotypes are Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Verrucomicrobia, Planctomycetes, and one archaeal taxa, Thaumarchaeota (Figure 3, Supplementary Figure S1). Acidobacteria, Bacteroidetes, and Proteobacteria have ubiquitous presence in soil, thus explaining the importance of these phyla in all our samples (Chowdhury et al. 2019; Sarkar et al. 2020). Similarly, Chloroflexi, Verrucomicrobia, Planctomycetes, and Thaumarchaeota are well-known soil dwellers, reported to contribute to diverse soil processes (Zhang, Chen, and Han 2013; Navarrete et al. 2015; Ivanova et al. 2016; Lu, Seuradge, and Neufeld 2017), which also explains the prevalence of the bacteria in our analyses regardless of the ecotypes. We used ANOVA followed by a Tukey post-hoc test in R (Team 2015), and found that Proteobacteria ($p = 0.004$) and Thaumarchaeota ($p = 0.020$) had statistically significant differences between the dry and wet ecotypes. Proteobacteria had a higher relative abundance in dry than in the wet ecotypes, while Thaumarchaeota showed the opposite (Figure 3). Proteobacteria have been identified to assist plant growth, and also have higher abundance under drought conditions (Jang et al. 2020; Kim et al. 2011), suggesting the important role that Proteobacteria might play towards the growth of *A.gerardii* during environmental challenging conditions in Colby. Thaumarchaeota were found to be the dominant archaea in soil systems (Schleper and Nicol 2010), and are well-known ammonia

oxidizers (Stieglmeier, Alves, and Schleper 2014). We surmise that Thaumarchaeota in our study had the potential to enhance the resilience of the *A.gerardii* wet ecotype under abiotic stressful conditions through the transformation of ammonia into nitrate (Taffner et al. 2018).

Post-hoc SIMPER analyses at the phylum level showed that Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes and Verrucomicrobia contributed to major similarities among the dry, mesic and wet ecotypes (Supplementary Table S3). We observed using post-hoc SIMPER analyses that Actinobacteria, Acidobacteria, Proteobacteria, Verrucomicrobia and Bacteroidetes contributed to the greatest differences between the dry and mesic ecotypes (Supplementary Table S3). Planctomycetes and Thaumarchaeota had contributions only in the mesic and wet ecotypes respectively (Supplementary Table S3). Comparing the dry and wet ecotypic rhizobiomes, we observed that Actinobacteria, Proteobacteria, Acidobacteria, Bacteroidetes, and Thaumarchaeota contributed to the highest differences. Actinobacteria, Proteobacteria, Acidobacteria, Verrucomicrobia, and Thaumarchaeota contributed most to the differences between the mesic and wet ecotypes.

We analysed the data with DeSEQ2 ($p < 0.05$, Figure 4) and detected that *Rhizobium* had significant differences between the dry and wet ecotypic rhizobiomes. *Rhizobium* had higher relative abundances in the dry ecotype. Comparing dry and mesic ecotypes, we noticed that *Rhizobium*, *Pseudomonas*, *Cellulomonas*, *Rhodococcus*, *Parviterribacter*, *Parasegetibacter*, *Flavihumibacter*, *Cellvibrio*, and *Candidatus Berkiella* were more dominant in dry ecotype than in mesic ecotype. On the opposite side, *Microbispora*, *Sorangium*, *Zavarzinella*, and *Candidatus Udaeobacter* had more dominance in mesic than in wet ecotype. In other studies, *Rhizobium* has been found to be drought-stress tolerant (Rehman and Nautiyal 2002), and well-known to aid plants during drought conditions (Staudinger et al. 2016). Putting it all together, our study

suggested that *Rhizobium,* being the most predominant in the dry ecotype, might have the potential influence to benefit the host in the dry environments. This may also help to explain the higher leaf nitrogen concentrations and higher chlorophyll absorbance we observed in the dry ecotype, regardless of planting location (Caudle et al.2014, Galliart et al. 2020). We compared the differences between mesic and wet ecotypes as well, and observed that *Parafrigoribacterium*, *Ktedonobacter*, *Streptosporangium*, *Acidicapsa*, and *Pseudoduganella* were more dominant in the mesic ecotype. On the contrary, the top genera that were more predominant in wet ecotypes as compared to the mesic belong to *Candidatus Berkiella*, *Cellvibrio*, *Flavihumibacter*, *Terrabacter, Parasegetibacter*, *Parviterribacter, Cellulomonas*, *Solitalea, Sphaerisporangium*, *Nonomuraea, Achromobacter*, *Acinetobacter, Pseudorhodoferax*, *Nocardia, Leucobacter,* among others (Supplementary Table S4). *Leucobacter* has been identified to grow in wet, low-light environments (Muir and Tan 2007), and we observed that *Leucobacter* had higher relative abundance in the wet ecotype (Muir Rachel E. and Tan Man-Wah 2008) suggesting that *Leucobacter* might be better adapted to the wet environment.

No differences in ecotypic fungi diversity and structure

Unlike the observed differences in rhizobiome bacterial diversity among ecotypes, we did not notice the same pattern in the ecotypic fungi diversity and structure. We observed that the ecotypic fungal communities structure were not significantly different from each other at the phylum level (PERMANOVA, Pseudo-F 2.0827, p (perm): 0.071, p (MC): 0.08). Pairwise Kruskal-Wallis test also indicated no significant differences in the rhizosphere fungal community composition between the ecotypes (dry v wet: p (MC): 0.190, dry v mesic: p (MC): 0.117, wet v mesic: p (MC): 0.072). The top eight fungal taxa present in all three ecotypes were Ascomycota, Basidiomycota, Mortierellomycota, Mucoromycota, Glomeromycota, Chytridiomycota, Kickxellomycota, and

Rozellomycota (Figure 5, Supplementary Figure S2). Besides the fact that Ascomycota and Basidiomycota had the highest relative abundance, post-hoc SIMPER analyses at the phylum level also indicated that Ascomycota and Basidiomycota were the top phyla contributing to the similarities in all the three ecotypes (Figure 5, Supplementary Table S3). Consistent with the results shown in our study, Ascomycota dominate the rhizosphere fungal communities (Egidi et al. 2019; Praeg and Illmer 2020; Qin et al. 2017). Basidiomycota has been reported to be isolated from soil as well, and is well-known for rhizobiome dwellers (Thorn et al. 1996; Yang et al. 2017). At the genus level, *Phallus* and *Cladosporium* (SIMPER analysis) were among the top genera contributing to the similarities between the ecotypes (Supplementary Table S3).

Ecotype differences in bacterial diversity evident under arid conditions

The distribution of microorganisms in an environment is often expressed in the famous tenet "everything is everywhere but the environment selects" (Baas Becking, 1934). Our ten-year-long reciprocal common garden study provided an excellent opportunity to gain insights into whether and how specifically adapted ecotypic microorganisms will thrive and proliferate under arid conditions. The ideal situation would be the ability to compare and contrast this study with the rhizobiome profiles before planting the *A. gerardii* ecotypes in Colby. After ten years of planting and maintaining under drought stress conditions, the environmental pressure would alter the rhizobiome structure, especially when the hosts or ecotypes had limited or no influences (Hueso, Hernández, and García 2011; Alster et al. 2013; Bachar et al. 2010; Fierer, Schimel, and Holden 2003). On the contrary, we observed that there were differences in bacterial community composition in perennial grassland ecotypes under drought stress, although there were no effects on the fungal compositions (Figure 2). Our study indicated that even under arid conditions, the

host-mediated adaptation persisted. The earlier report associated with *A. gerardii* has indicated that the ecotypic variation was observable in several aspects of growth in the plant. The ecotypic variation not only impacted aboveground features, but also had a prominent effect on the belowground ecosystem processes mediated by microbial communities (Mendola et al. 2015). We observed the higher relative abundance of *Rhizobium* in the dry ecotypic rhizosphere, suggesting that the *Rhizobium* might be associated with the observed higher leaf nitrogen concentrations and chlorophyll absorbance (Caudle et al.2014, Galliart et al. 2020). The plant host can implement diverse mechanisms by secreting root exudates, employing defense strategies and structural modifications to recruit a specific and optimized microbiome (Pascale et al. 2019). We demonstrated in our study that the individual plant genotype might influence the bacterial rhizobiome, and other reports showed that these traits could also be heritable (Schweitzer et al. 2008; Peiffer et al. 2013; Walters et al. 2018; Gray et al. 2014). There is a fine margin between the influence by the ecotype host and the environment on the microbiome communities. In our present work, we were able to determine that even after ten years of growing under extreme drought, the host ecotype might be able to "overcome" environmental pressure to a certain extent and regulate the bacterial community composition. Previous studies have indicated that drought stress can escalate the relative abundance of fungal populations while decreasing the relative abundance of bacterial communities in the same poplar plantation (Sun et al. 2020). Also, it is a well-known fact that bacterial networks in soil are less stable than the fungal networks (de Vries et al. 2018). Putting all these together, our study suggested that the ecotypic fungal populations might be more capable at adapting, and could have diverse resistance mechanisms towards drought conditions, and therefore the fungal community composition and structure remain unchanged.

In this study, we explored the concept of microbial “generalists" and “specialists”. Insights into the β-Diversity analysis of the ecotypic rhizobiome suggested that the bacterial population from the dry and wet ecotypes might be specialists and are less adaptive to the induced environmental stress. We further hypothesized that the bacterial populations from the mesic ecotypes belonged to the generalist group, which strived to adapt to the drought environment. Generalist microbial populations are able to adapt to diverse habitats, while microbial populations are referred to as the specialists when those can only adapt to specific habitats (Sriswasdi, Yang, and Iwasaki 2017). There are previous reports which acknowledge the contribution of the generalists and specialists impacting the dynamics of the structures of different microbial communities (Pandit, Kolasa, and Cottenie 2009; Székely and Langenheder 2014). We observed that the differences in bacterial community structures were more prominent between dry and mesic as well as wet and mesic, as compared to dry and wet ecotypes (Figure 2). Putting this all together, our study suggested that dry and wet ecotypic microbial communities might show more influences from the host as compared to that of the mesic ecotypes. From this study, we surmised that *A. gerardii* ecotypes had unique bacterial assemblage contributing to the rhizobiome. However, the classification of whether the bacterial populations were generalists or specialists would influence the resultant ecotypic rhizobiome due to host and environmental interaction.

To conclude, our study provided the knowledge that will help tackle the challenges faced by grassland restorative efforts by providing insights into the impact of environmental stress on plant host-associated microbiomes. We showed that bacterial populations were influenced by the respective ecotypes, while the fungal populations were not significantly different between the ecotypes. This study also suggested the existence of host-mediated bacterial community adaptation

for *A.gerardii* rhizosphere, and a possible trade-off between the specialist and generalist bacterial communities in specific environments, that might ultimately lead to the benefit of the plant host.

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Figure legends:

Figure 1A: Bacterial α-*Diversity* indices among the dry, mesic, and wet ecotypes. (a) Shannon index (b) faith-pd index and (c) observed OTUs index (d) Venn diagrams representing the overlapping bacterial and archaeal ASVs among the dry, mesic, and wet rhizobiomes. The bacterial α-*Diversity* was not significantly different among the samples.
Figure 1B: Fungal α-*Diversity* indices among the dry, mesic, and wet ecotypes. (a) Shannon index (b) faith-pd index and (c) observed OTUs index (d) Venn diagrams representing the overlapping fungal ASVs among the dry, mesic, and wet rhizobiomes. The fungal α-*Diversity* was not significantly different among the samples.

Figure 2: Bacterial and fungal *β-Diversity* between the dry, mesic, and wet ecotypes. NMDS ordinations were obtained from Bray-Curtis similarity matrix. The matrix was calculated from square-root transformed relative abundance of 16S and ITS rRNA sequences (A) bacterial community compositions are separated between the three ecotypes (B) fungal community compositions are not separated between the three ecotypes.

Figure 3: The relative abundance of the top seven bacterial and one archaeal taxa present in all the three ecotypes. Proteobacteria and Thaumarchaeota were significantly different between the dry and wet ecotypes. Letters in a box plot are significantly different at $p < .05$ (D, M, W - significantly different from dry, mesic and ecotypes respectively).

Figure 4: DeSEQ2 analysis to reveal the bacterial genera that were significantly different in relative abundance between the dry, mesic, and wet ecotypes ($p < 0.05$). Pairwise comparisons were performed between wet-mesic, dry-mesic and dry-wet ecotypes.

Figure 5: The relative abundance of Ascomycota and Basidiomycota in the dry, mesic and wet ecotypes. Ascomycota and Basidiomycota are also the most abundant phyla in all the ecotypes.

Supplementary Files:

Supplementary Table S1: Raw sequence analysis by QIIME 2 Version 2019.7. The number of counts of bacteria and fungi initially obtained, and the counts that were considered after primer trimming and DADA2 quality control per sample.

Supplementary Table S2: Bacterial and fungal identifications along with the number of counts per sample.

Supplementary Table S3: Post-hoc SIMPER analyses indicate the bacterial and fungal phyla that contributed to major similarities among the dry, mesic, and wet ecotypes.

Supplementary Table S4: Genera that had differential relative abundance between dry, mesic and wet ecotypes.

Supplementary Figure S1: The top bacterial and archaeal taxa present in dry, mesic and wet ecotypes.

Supplementary Figure S2: The top fungal taxa present in dry, mesic and wet ecotypes.

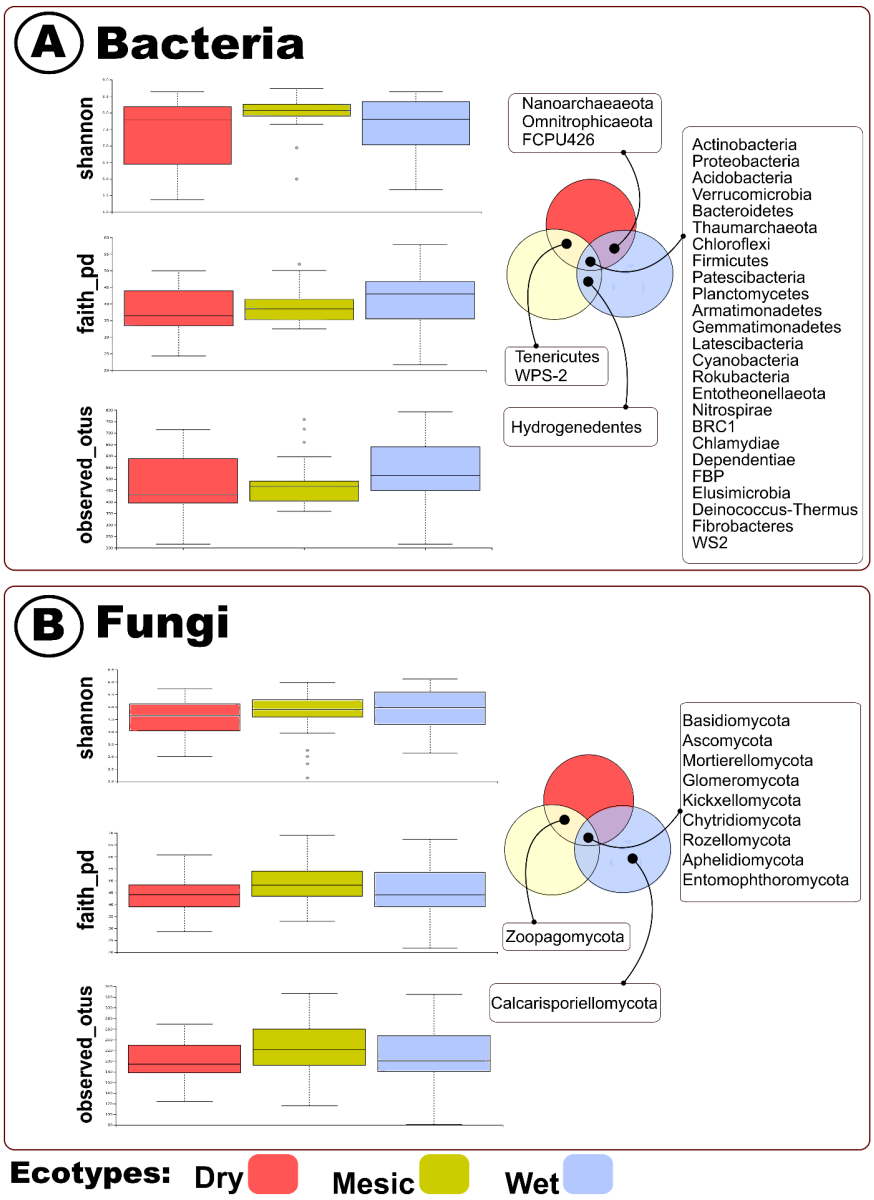
A Bacteria
shannon
faith_pd
observed_otus
Nanoarchaeaeota
Omnitrophicaeota
FCPU426
Tenericutes
WPS-2
Hydrogenedentes
Actinobacteria
Proteobacteria
Acidobacteria
Verrucomicrobia
Bacteroidetes
Thaumarchaeota
Chloroflexi
Firmicutes
Patescibacteria
Planctomycetes
Armatimonadetes
Gemmatimonadetes
Latescibacteria
Cyanobacteria
Rokubacteria
Entotheonellaeota
Nitrospirae
BRC1
Chlamydiae
Dependentiae
FBP
Elusimicrobia
Deinococcus-Thermus
Fibrobacteres
WS2
B Fungi
shannon
faith_pd
observed_otus
Basidiomycota
Ascomycota
Mortierellomycota
Glomeromycota
Kickxellomycota
Chytridiomycota
Rozellomycota
Aphelidiomycota
Entomophthoromycota
Zoopagomycota
Calcarisporiellomycota
Ecotypes: Dry Mesic Wet

Figure 1

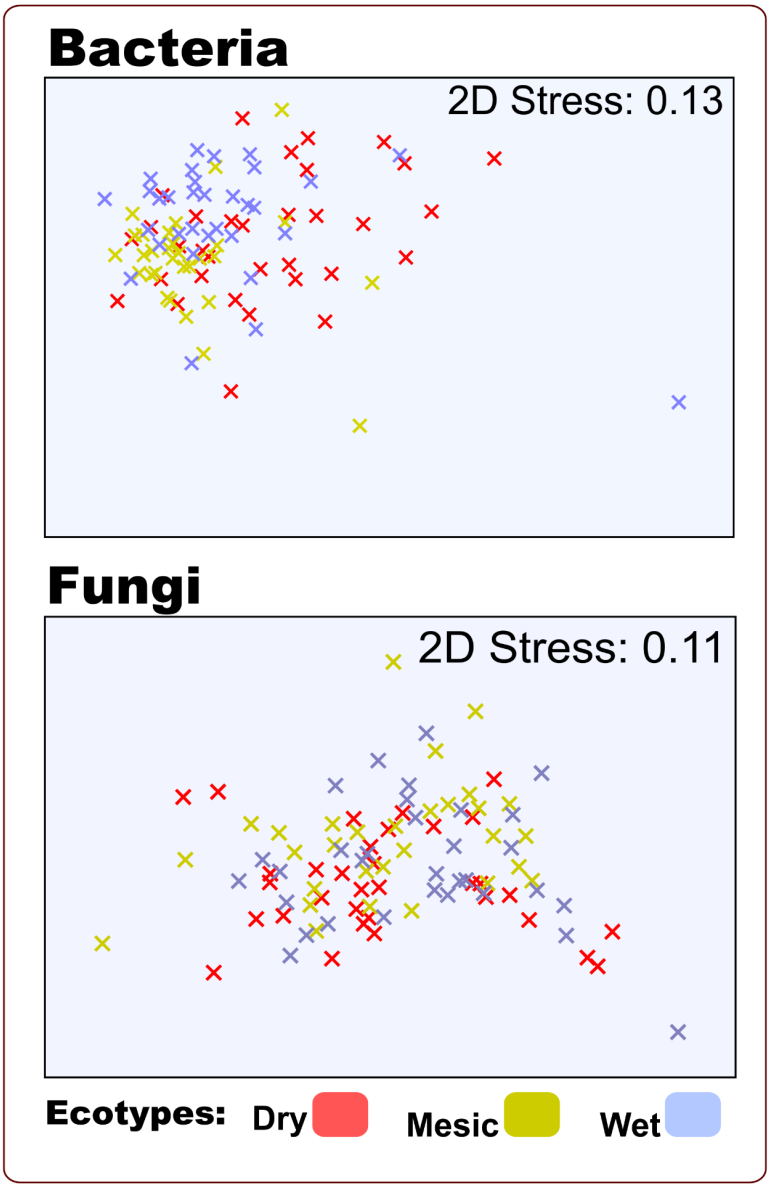
Bacteria
2D Stress: 0.13
Fungi
2D Stress: 0.11
Ecotypes:
Dry
Mesic
Wet

Figure 2

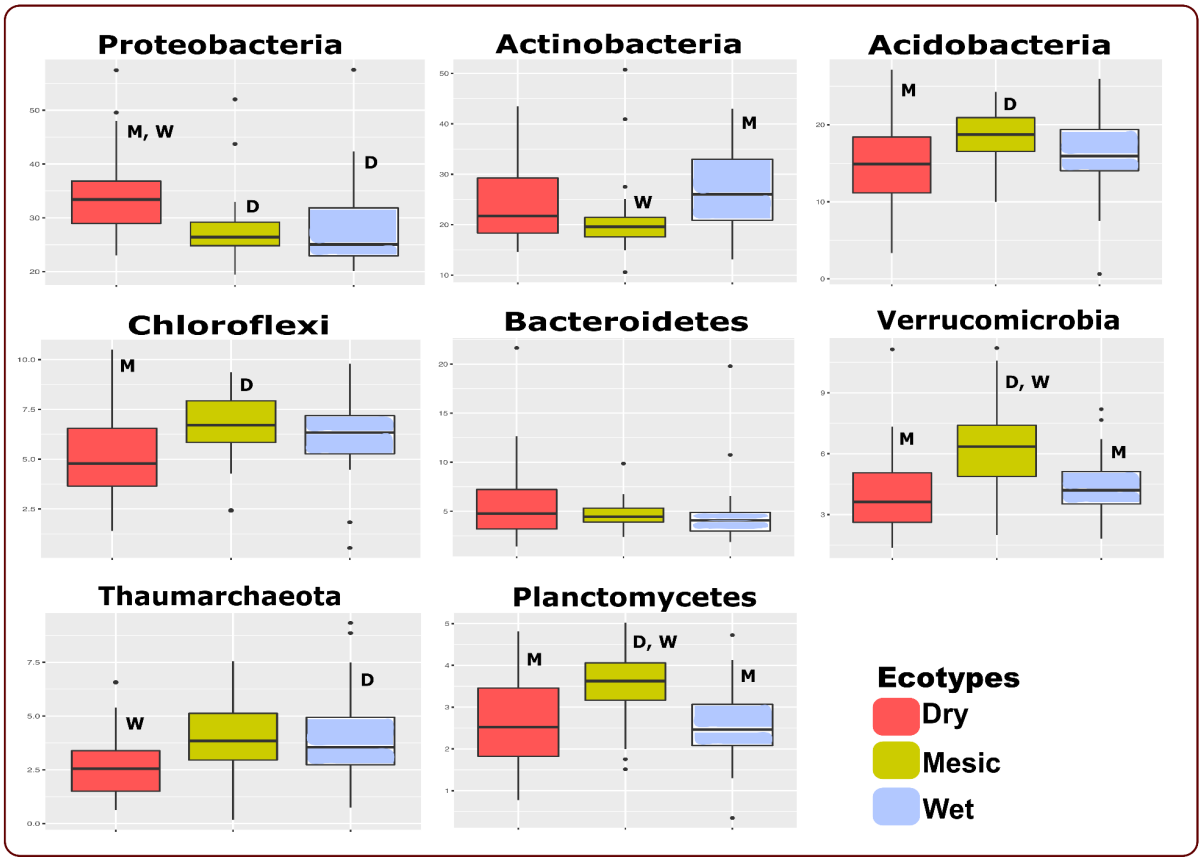
Proteobacteria
M, W
D
D
Actinobacteria
W
M
Acidobacteria
M
D
Chloroflexi
M
D
Bacteroidetes
Verrucomicrobia
M
D, W
M
Thaumarchaeota
W
D
Planctomycetes
M
D, W
M
Ecotypes
Dry
Mesic
Wet

Figure 3

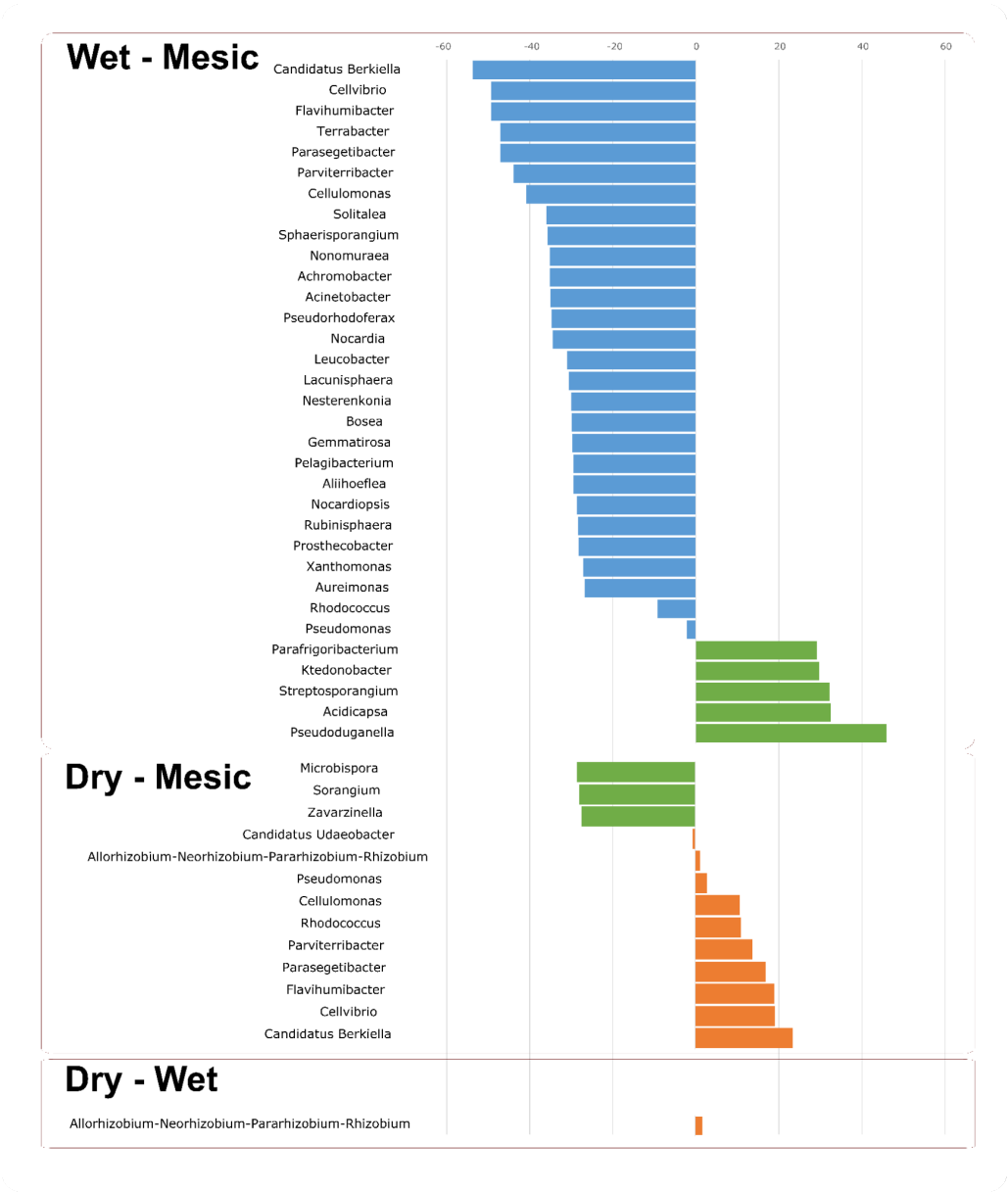
Wet - Mesic
-60
-40
-20
0
20
40
60
Candidatus Berkiella
Cellvibrio
Flavihumibacter
Terrabacter
Parasegetibacter
Parviterribacter
Cellulomonas
Solitalea
Sphaerisporangium
Nonomuraea
Achromobacter
Acinetobacter
Pseudorhodoferax
Nocardia
Leucobacter
Lacunisphaera
Nesterenkonia
Bosea
Gemmatirosa
Pelagibacterium
Aliihoeflea
Nocardiopsis
Rubinisphaera
Prosthecobacter
Xanthomonas
Aureimonas
Rhodococcus
Pseudomonas
Parafrigoribacterium
Ktedonobacter
Streptosporangium
Acidicapsa
Pseudoduganella
Dry - Mesic
Microbispora
Sorangium
Zavarzinella
Candidatus Udaeobacter
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Pseudomonas
Cellulomonas
Rhodococcus
Parviterribacter
Parasegetibacter
Flavihumibacter
Cellvibrio
Candidatus Berkiella
Dry - Wet
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium

Figure 4

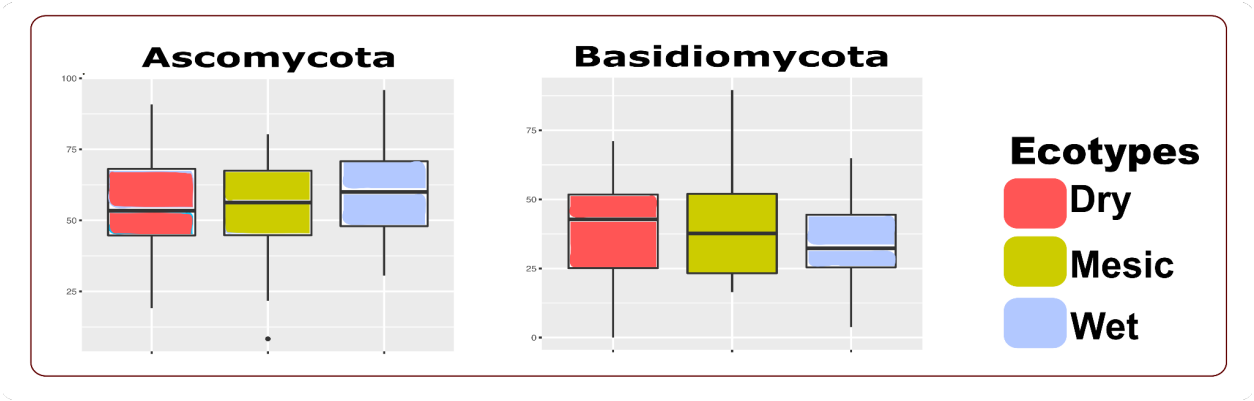
Ascomycota
Basidiomycota
100
75
50
25
75
50
25
0
Ecotypes
Dry
Mesic
Wet

Figure 5